AD-A160 668 MEETING OF THE INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY (10TH) HELD AT RI.. (U) OFFICE OF NAVAL RESEARCH LONDON (ENGLAND) C E ZOMZELY-NEURATH 08 OCT 85 ONRL-C-10-85 1/1

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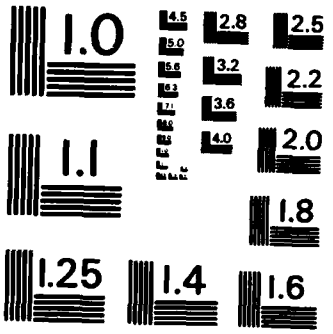

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ONRL Report C-10-85

AD-A160 668

10th Meeting of the International Society for Neurochemistry

Claire E. Zomzely-Neurath

8 October 1985

U.S. Office of Naval Research, London

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

AD-A166668

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b DECLASSIFICATION/DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) C-10-85	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION US Office of Naval Research Branch Office, London 6b OFFICE SYMBOL (If applicable) ONRL	7a NAME OF MONITORING ORGANIZATION
6c ADDRESS (City, State, and ZIP Code) Box 39 FPO, NY 09510	7b ADDRESS (City, State, and ZIP Code)
8a NAME OF FUNDING/SPONSORING ORGANIZATION 8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c ADDRESS (City, State, and ZIP Code)	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO / PROJECT NO / TASK NO / WORK UNIT ACCESSION NO

11 TITLE (Include Security Classification)
10th Meeting of the International Society for Neurochemistry

12 PERSONAL AUTHOR(S)
Claire E. Zomzely-Neurath

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Conference	FROM _____ TO _____	8 October 1985	10

16 SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Neurochemistry
Neurobiology
Italy

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The Tenth Meeting of the International Society for Neurochemistry was held in Riva del Garda, Italy, from 19 through 24 May 1985. This report discusses presentations on molecular neurobiology, post-translational modification, neurotransmitter receptors, neuropeptide processing, and specific macromolecules in cell-cell interactions in the nervous system.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a NAME OF RESPONSIBLE INDIVIDUAL Larry E. Shaffer	22b TELEPHONE (Include Area Code) (44-1) 409-4340 22c OFFICE SYMBOL 11

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

10TH MEETING OF THE INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY

The Tenth Meeting of the International Society for Neurochemistry (ISN) took place at Riva del Garda, Italy, from 19 through 24 May 1985. The total attendance was about 900, with 780 preregistrations. The ISN meetings take place every 2 years in different countries as the membership is truly international in scope.

Although the US scientists account for 46 percent of the total membership of the ISN, the US representation at this meeting accounted for 27.5 percent of the total number of participants, with Western European countries contributing 53.3 percent. Scientists from Italy, France, West Germany, and the UK were the largest groups from the Western European countries, about twice the representation in the membership of the ISN. This means that there were a sizable number of non-ISN member attendees. The Eastern European countries accounted for 4.5 percent of the total number of participants, about twice as many as at past ISN meetings held in Western European countries.

Representation by Japanese scientists was very high, 7.5 percent of the total. This large attendance by Japanese scientists is a recent phenomenon and has been reflected in their attendance at European meetings in all areas of scientific research, including biotechnology, pharmacology, etc.

The scientific program consisted of 10 symposia covering a wide range of topics in neurobiology (Table 1), as well as oral communications, poster sessions, and round tables (workshops).

The most striking change in the topics covered at this ISN meeting was the number of sessions on the use of molecular biological methods including recombinant DNA (rDNA)--i.e., molecular neurobiology) in neurochemical studies. Many of the speakers were scientists whose training and experience were in molecular biology and who are now using the powerful techniques of rDNA to resolve problems in neurobiology that either cannot be answered or can be answered only with great difficulty by the usual neurochemical methods.

The subject of neuronal membranes, encompassing studies of ion channels and lipid metabolism, was also emphasized in the presentations. Sessions on neurotransmitter receptors, and studies of glial cell function and neuropeptides were more prominent than at previous meetings of the ISN. It was also evident from the presentations that tissue culture and immunological methods are being used more often in neurobiological studies.

It is only possible to present selected topics in this report due to the large number of topics and sessions. Emphasis is given to research presented by European scientists since US readers are more familiar with the research in the US.

Abstracts of the ISN meeting have been published in the *Journal of Neurochemistry*, Vol 44 supplement (1985) by Raven Press, New York; this is the official journal of the ISN.

Molecular Neurobiology: The Impact of Molecular Genetics on Neurochemistry

J. Mallet (Centre National de la Recherche Scientifique [CNRS], Gif sur Yvette, France) gave an excellent presentation of his research on catecholamines using a molecular genetic approach. Catecholamines represent an important class of neurotransmitters, and they constitute a useful model for the analysis of the mechanisms involved in the regulation of gene expression during development and during the normal functioning of the mature nervous system.

Mallet and his coworkers have constructed recombinant DNA plasmids that contain structural sequences for rat tyrosine hydroxylase (TH), the enzyme that catalyzes the rate-limiting step in the biosynthesis of catecholamines. The complete sequence of rat TH enzyme was deduced from the analysis of complementary (cDNA) clones. The TH probe was used to analyze the transcriptional and

Table 1

Symposia and Colloquia

Symposia

1. The Impact of Molecular Genetics in Neurochemistry
2. Neuronal Membranes: The Modulation of Lipid Metabolism and its Consequences
3. Functional Implication of Post-translational Modification in the Nervous System
4. Neuronal Membranes: The Role of Ion Channels
5. Neurotransmitter Receptors: New Approaches
6. Mechanisms of Axonal Transport
7. The Aging Brain
8. Molecular Aspects of Neural Development and Plasticity
9. Neuropeptide Processing
10. Specific Macromolecules in Cell-Cell Interactions in the Nervous System

Colloquia

1. Molecular Events Underlying Simple Learning and Memory Events
2. Prostaglandins and Leukatrienes in Brain Function
3. Dynamic Aspects of Transmitter Storing Organelles
4. Recent Developments in the Study of Glial Cells
5. The Future for the Brain Graft
6. Environmental Neurotoxic Substances

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post-transcriptional regulation of TH gene expression during development and electrical activity of catecholaminergic neurons. Mallet et al. have carried out preliminary studies of the localization of TH mRNA by *in situ* hybridization on tissue sections. Mallet et al. have also isolated the TH nuclear gene from a rat cosmid library. Its structure has been analyzed, and it has been used for the cotransformation of various cell lines. Transcription and translation of the TH gene occurred in the transfected cells. This approach will be most useful in assessing the functional role of sequences preceding the coding portion of the gene. Furthermore, Mallet et al. have isolated the human TH gene using their rat probe and have localized it to the short arm of chromosome 11. These investigators are extending their research to other neurotransmitter synthesizing enzymes and presented preliminary data on the dopamine-β-hydrolyase (DBH) gene. In addition, Mallet et al. have initiated investigations of TH gene polymorphism using restriction enzymes.

S. Numa (Department of Medicinal Chemistry, Kyoto University Faculty of Medicine, Kyoto, Japan) presented the plenary lecture at the ISN meeting. His lecture dealt with structural and functional studies on the nicotinic acetylcholine receptor using molecular cloning techniques. Numa is one of the foremost investigators in this area of research. The nicotinic acetylcholine receptor and the sodium channel represent ligand-gated and voltage-gated channel proteins, respectively, which mediate neural signaling by modulating the ion permeability of electrically excitable membranes. Numa and his group have deduced the primary structures of all four subunits of the acetylcholine receptor from *Torpedo californica* electroplax as well as from mammalian skeletal muscle from the cDNA or genomic DNA sequences. They found that the four subunits exhibited marked amino acid sequence hemology and are similar in hydrophilicity profile and predicted secondary structure, thus being oriented most probably in a pseudosymmetric fashion across the membrane. The functional regions of the acetylcholine receptor, including the channel forming region, were located by means of site-directed mutogenesis of the α-subunit cDNA combined with expression in *Xenopus* oocytes.

Numa et al. have also deduced the primary sequence of the sodium channel from *Electrophorus electricus* electroplax from the cDNA sequence. This protein has four internal repeats showing sequence hemology, which are oriented presumably in a pseudosymmetric fashion

across the membrane. Each repeat contains a unique segment with clustered, positively charged amino acid residues, which may be involved in the voltage-dependent gating of the channel, possibly in conjunction with negatively charged residues clustered elsewhere, according to Numa et al.

These elegant studies by Numa and his group are a major contribution to our knowledge about acetylcholine receptors and sodium channels, which play a major role in nervous system function.

D.W. Schmid (Department of Neurochemistry and Biophysical Chemistry, University of Göttingen, West Germany) reported on the selection of clones representing sequences probably connected with the regulation, function, and differentiation of cholinergic synaptic transmission by using differential hybridization. Messenger RNA was isolated from the purely cholinergic electric lobes of *Torpedo marmorata* and used to prepare the cDNA. The cDNA was rendered double-stranded and incorporated into pBR 3222 plasmids by G/C hybridization. Competent *E. coli* K12 cells were transfected with the hybrid plasmids. Tetracycline-resistant, ampicillin-sensitive clones were transferred to nitrocellulose filters and used for *in situ* colony hybridization with the following radioactively labeled cDNA probes: (1) electric lobe (positive control), (2) cerebellum (selection), and (3) muscle (non-neuronal control). This method selects for sequences involved in the cholinergic function of electric lobe perikarya as the cerebellum has few or no cholinergic synapses. Therefore, a cerebellar cDNA probe should not hybridize with cholinergic specific sequences. Schmid obtained 48 clones from 3200 clones by a differential hybridization method. These clones are now being studied in detail.

A report by a research group at the Neurobiology Laboratory, La Trobe University, Bundvora, Australia (O. Bernard, C.C.A. Bernard, and P. Bartlett) showed the power of recombinant DNA techniques to obtain a novel, highly basic neuronal protein which could not have been found by any other method. These investigators used an approach designed to characterize protein and mRNA expressed in the brain. They constructed an expression cDNA library from fetal calf mRNA using the expression vector λ gtll Amp 3. Following screening of this library with polyclonal antibodies of myelin basic protein (MBP), several clones were isolated. One of these, designated P_3, was found to be brain-specific but not species-specific. It hybridized to restriction fragments obtained from calf, human, and mouse DNA. Sequence analysis of P_3 revealed that although this clone was isolated using anti-MBP, its sequence was unrelated to that of MBP. *In situ* hybridization using this cDNA probe on brain slices showed that the mRNA is predominately expressed in the frontal cortex, cerebellum, hippocampus, and the ventral tegmental area. Further *in situ* hybridization studies performed on isolated brain cells indicated that this mRNA is only expressed in spinal cord neurons but not oligodendrocytes or astroglia (glial cells) as in the case for MBP. The investigators are continuing their studies of this novel basic neuronal protein to ascertain its role in nervous system function.

A. Guiditta (Institute for General Physiology, Naples, Italy) reported at the molecular biology symposium on an intriguing phenomenon found in squid axoplasm. This research was a cooperative project with B.B. Kaplan of the Western Psychiatric Institute, Pittsburgh, Pennsylvania. These investigators obtained mRNA from isolated giant squid axoplasm. This was a novel finding since axons and nerve endings are generally believed to lack the capacity to synthesize proteins and therefore should not contain mRNAs, which code for protein synthesis. Subcellular fractionation studies indicated that the axoplasm mRNA is largely present in the microsomal fraction and that this mRNA differed from giant fiber lobe mRNA (neuronal perikarya, the major site of protein synthesis in eukaryotes) and axonal sheath mRNA (periaxonal glia). These investigators prepared cDNA probes,

hybridizing them with poly (A)+ mRNA from squid brain. They compared the sequence complexity of axoplasmic mRNA with that of the giant fiber lobe and brain. Their results indicate that the complexity of axoplasmic mRNA is at least tenfold lower than brain mRNA and slightly lower than giant fiber lobe mRNA. The data suggest that the high degree of complexity of squid brain mRNA is largely due to the cellular and subcellular heterogeneity of brain tissue as contrasted with much less complexity of axoplasm. The axoplasm contains a heterogeneous family of mRNAs, and further studies are being carried out to separate the mRNAs and to prepare the respective individual cDNA probes for characterization. With these probes, it will be possible to find out if mammalian axoplasm also contains mRNAs which code for proteins that may play an important role in axoplasmic function.

Post-translational Modification: Functional Implications

M.B. Kennedy (California Institute of Technology, Pasadena, California) presented an overview of calcium-dependent protein phosphorylation in the nervous system and discussed his current research. It is now clear from the work of many labs that calcium-dependent protein phosphorylation plays an important role in neuronal responses to changes in internal calcium concentration. Several distinct calcium-dependent protein kinases have been identified in the nervous system, including the calcium- and lipid-dependent C-kinase and various types of Ca^{2+}/calmodulin-dependent protein kinases.

Kennedy and his group have carried out studies to learn how these kinases are distributed in neurons in different brain regions and in subcellular structures within these neurons in order to understand the role that these kinases and their substrates play in determining the nature of specific responses to calcium ion. A class of kinases called type II Ca^{2+}/calmodulin-dependent protein kinases are particularly abundant in brain. Radioimmunoassays and immunohistochemical studies by Kennedy et al. indicate that the brain type II calmodulin-dependent kinase is much more highly expressed in telencephalic neurons than in neurons in lower brain regions. For example, it comprises 1 to 2 percent of the total protein in the hippocampus, compared to 0.05 percent of the total protein in the pons/medulla. It thus appears, according to Kennedy, that this kinase activity is an important part of specific responses of telencephalic neurons to calcium. In addition, approximately half of the type II calmodulin-dependent kinase in brain homogenates was found to be soluble. Another portion is a major component of the postsynaptic density fraction, and the rest appears to be loosely associated with cytoskeletal structures. The hypothesis was presented that these calcium-dependent protein kinases may play a role in neuronal modulation in learning and memory.

P. De Camilli (Consiglio Nazionale delle Richerche, Center of Cytopharmacology and Department of Medical Pharmacology, University of Milan, Italy) discussed the importance of the immunohistochemistry approach as a tool in the study of the function of phosphoproteins. This research area is progressing rapidly and is very important as phosphorylation-dephosphorylation of proteins appears to be one of the major mechanisms by which intracellular second messengers regulate neuronal function. De Camilli presented data on two major phosphoproteins which have a widespread distribution throughout the neuronal population, Synapsin I and MAP_2. Synapsin I is a nerve-cell-specific phosphoprotein which is involved in a secretory pathway specific to neurons. MAP_2 is a microtubule-associated protein selectively concentrated in perikarya and dendrites. It appears to mediate actions of neurotransmitters on the dendritic cytoskeleton.

De Camilli also presented data on a project carried out with P. Greengard (Laboratory of Cellular and Molecular Neuroscience, Rockefeller University, New York) on the membrane protein,

Synapsin I (SYNI) and a new protein, P36. They found that SYNI, a major phosphoprotein of nerve endings, is specifically associated with small synaptic vesicles (SSV), but it is absent from large dense-core vesicles (LDCV) and secretory granules of endocrine cells. By contrast, P36, associated with the membrane of SSV, is also present on the membrane of endocrine granules and of LDCV. The selective association of SYNI but not of P36, with the membrane of SSV indicates that SYNI is involved in a secretory pathway specific to neurons. A unique property of SSV is their ability to undergo a local exoendocytotic cycle in nerve endings. SYNI might therefore be involved in regulating some aspects of this local traffic.

N. Murray and A.J. Steck (Department of Neurology, University of Lausanne, Switzerland) reported on studies of protein kinase C and myelin basic protein (MBP) phosphorylation. The involvement of Ca^{2+}-dependent protein kinase in MBP phosphorylation has long been suspected since in isolated myelin, MBP phosphorylation is stimulated by Ca^{2+}. These investigators have identified this kinase as a phospholipid-sensitive, Ca^{2+}-dependent protein kinase (protein kinase C). Protein kinase C is thought to be activated *in vivo* by diacylglycerol transiently produced by the breakdown of phosphatidylinosital, rather than by changes in intracellular Ca^{2+}. Thus the involvement of this kinase in MBP phosphorylation is of considerable interest for understanding the mechanisms regulating MBP phosphorylation. Murray and Steck are currently engaged in studies to define the role of this enzyme in depolarization-induced MBP phosphorylation in intact optic nerve.

P.F. Maness (Department of Biochemistry, University of North Carolina, School of Medicine, Chapel Hill) reported on studies of normal cellular SRC protein in the developing nervous system. This protein is very interesting in that it is the normal cellular homologue of Rous Sarcoma virus, and the gene coding for this protein is called an oncogene--i.e., under certain conditions results in malignancy. Maness used the developing chick retina for her studies and found that the SRC protein, termed pp60 C^{-SRC}. was expressed at elevated levels in the embryonic nervous system. Data were presented showing that this protein was the product of neurons appearing at the onset of cell differentiation when cell proliferation ceases. In further studies, pp60 C^{-SRC} was also localized to the cerebellum during neurogenesis. In both the retina and cerebellum this protein was most abundant in regions rich in cell processes and was absent from the cell bodies of most cerebellar and retinal neurons at all stages of development. The amount of protein relative to DNA content in brain and neural retina increased during the embryonic-to-adult transition as the neuronal processes grew, suggesting a possible role in mature neuronal function. There appear to be two developmental phases of pp60 C^{-SRC} expression: one associated with early embryonic events, and the other with a mature neuronal function. However, the specific function of this SRC protein is as yet unknown, and studies are being carried out to define the function.

W. Huttner and coworkers (Max Planck Institute for Psychiatry, Martinsried, West Germany) presented a symposium lecture on studies of tyrosine sulfation of proteins in neuronal and nonneuronal cells. Proteins containing sulfated tyrosine residues have been found to occur in all multicellular invertebrate and vertebrate animals studied. The proteins have been detected in a variety of tissues. Despite this widespread occurrence, all these proteins characterized so far belong to one specific class of protein: the secretory proteins. Huttner et al. hypothesized that the primary function of tyrosine sulfation was to control some presecretion event (or events), such as the intracellular sorting of certain secretory proteins. In a neuronal model system, the chick retinotectal pathway, the major tyrosine-sulfated proteins were

indeed found to be sorted into membrane vesicles destined to reach the nerve terminals in the optic section by fast axoplasmic transport. Data by Huttner et al. indicated that the tyrosine-sulfating enzyme might be located in the trans-Golgi or an early post-Golgi compartment. These investigators theorize that the ways in which tryosine sulfation may be involved in the sorting of secretory proteins include the following: (1) sulfated tyrosine residues of certain secretory proteins may constitute a recognition marker that could be recognized by a tyrosine sulfate receptor protein; (2) tyrosine sulfation may lead to exposure of other domains of secretory proteins that serve as recognition markers; (3) sulfation of tyrosine residues may induce the dissociation of certain secretory proteins from receptors to which these proteins were bound during their intracellular transport. Huttner et al. are continuing their studies of tyrosine sulfation of proteins to try to find the specific functions, particularly in the nervous system, where their role may prove to be very important in nervous system function.

Neurotransmitter Receptors: New Approaches

The presentations in this symposium dealt essentially with approaches which lead to a better molecular understanding of neurotransmitter receptors and cellular excitability at the molecular level.

J.L. Barker (Laboratory of Neurophysiology, NINCDS, National Institutes of Health, Bethesda, Maryland) reported on receptor regulation of excitable membrane properties in cultured primary central nervous system (CNS) neurons and clonal pituitary cells. He used quantitative electrophysiological techniques to study the chemical excitability in these preparations. Since virtually every central neuron responds to GABA (γ aminobutryic acid), he and his group focused on Cl^--dependent signals transmitted by GABA. GABA activates microscopic, unitary steps in Cl^- conductance. Evoked release of GABA at synapses simultaneously activates several thousand steps to generate the signal peaks. The exponential distribution in step durations determines the envelope and the millisecond time course of signal decay. Glycine-activated steps are noticeably shorter than those induced by GABA, and so are the synaptic signals. Thus, each transmitter mediates a unique inhibitory event. Barker et al. also found that pharmacological applications of TRH (thyrotorpin-releasing hormone) to clonal pituitary cells showed that this peptide has multiple long-lasting actions on electrical excitability, most of which are likely to be derived from a triggered release of calcium from intracellular stores. This neurohormonal form of communication is thus distinctly different from that seen at synapses.

E. Barnard et al. (Department of Biochemistry, Imperial College, London) have applied the techniques of molecular genetics to receptor analysis in their studies of the nicotinic acetylcholine (Ach) and GABA receptors. They have isolated mRNAs and cloned genes for polypeptides of these two receptor systems. Purification of the protein was followed by determination of the subunit structure and of subunit protein sequences as well as antireceptor antibody production. These approaches provided tools for screening cloned receptor cDNAs. Barnard et al. have achieved translation and assembly of functional receptor/ion channel systems for both Ach and GABA in the mRNA-injected *Xenopus* oocytes. This approach is being used in devising cloning protocols and in studies of receptor mRNAs in tissue to obtain detailed information on the structure and function of neurotransmitter receptors.

M.M.S. Lo (Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland) and his group have recently developed a new method for the production of B-cell hybridomas. It is based on the specific contact obtained between B-cells and biotinylated myeloma cells when they are cross-linked with a chemical conjugate

of the antigen and avidin. Cell pairs are fused efficiently in an intense electric field, and the hybrids are subsequently selected by conventional means. The significant advantages of this process are: (1) antibodies formed have very high binding affinities (typically K_D $<10^{-9}$ to 10^{-11}M); (2) most hybridomas recovered produce antibodies of interest, thus greatly reducing the screening involved; and (3) the efficiency and sensitivity of this method provides significant improvements in cases where only a minute amount of the antigen is available. In addition, antibodies can be produced to antigen present in a crude form when its purification is not possible. Studies of receptor localization and function as well as the production of antibodies to new receptors and to those for which antibody is not available at present should be greatly facilitated by the use of the technique of Lo et al.

J. Kirilovsky, S. Steiner, and M. Schramm (Department of Biological Chemistry, The Hebrew University, Jerusalem, Israel) analyzed the role of lipids in the interaction of the β-adrenergic receptor (β-R) with the regulatory protein (Gs). These two components were delipidated, and the requirement of specific lipids in reconstitution of this β-adrenergic system was investigated. These investigators found that a mixture of phosphatidylethanolamine, -serine, and -choline plus α-tocopherol efficiency reconstituted the β-RG_s system. These findings should facilitate studies of structure/function relationships of the β-adrenergic system.

Neuropeptide Processing

This important topic in neurobiology was covered in an excellent symposium at the ISN meeting. It is now evident that most bioactive neuropepetides are derived from precursor molecules (polyproteins). Therefore, studies of processing as well as of the precursors can result in the discovering of novel neuropeptides and provide information on the biological roles of precursor and derived peptides.

E. Herbert (Department of Neuroscience, University of Oregon, Eugene, Oregon), well known for his research on opioid peptides, presented a very interesting lecture on the regulation of expression of opioid peptide genes in different species. Past research by Herbert and his group as well as other researchers has shown that all of the 15 opioid peptides reported to date are derived from three different precursors: preopiomelanocortin (POMC), proenkephalin A (proenkephalin), and proenkephalin B (prodynorphin). The genes that code for these precursors have been isolated and sequenced. Herbert et al. are studying the regulation of expression of the opioid peptide genes by gene transfer methods. For example, human proenkephalin gene has been transferred into frog oocytes and AtT-10 cells (mouse pituitary tumor cells that synthesize POMC but not proenkephalin). Both of these recipient cells express transfected gene. Several permanently transformed AtT-20 clones have been isolated that express human proenkephalin mRNA at a level comparable to that of POMC mRNA (about 1 percent of total mRNA). These clones synthesize and process proenkephalin predominantly to metenkephalin. Proenkephalin in mRNA, like POMC mRNA expression, has been found to be under control of secreting agents in these transformed clones--e.g., corticotropin releasing factor, cyclic AMP, phorbol esters, and glucocorticoids. Herbert et al. are determining the sequences in the transfected gene that are required for response to these agents by *in vitro* mutagenesis techniques. Production of bioactive peptides from polyproteins such as the precursors to the opioid peptides involves endoproteolytic cleavage at basic amino acid residues in the precursor followed by the action of carboxypeptidases. Herbert et al. have identified a kallikrein enzyme (member of a large family of trypsin-like serine proteases), a thiol protease, and a carboxypeptidase in AtT-20 cells, and these enzymes have been implicated in the processing of POMC. The amino acid sequence of the kallikrein enzyme, determined

by recombinant DNA methods, was proved to be different from any other kallikrein enzyme previously discovered in the mouse. The kallikrein mRNA was shown to be present in the neural intermediate lobe (the most active site of POMC synthesis) of mouse pituitary and in the mouse hypothalamus. Herbert et al. are using gene transfer techniques to determine if this kallikrein enzyme is required for POMC processing.

B.R. Seizinger (Max Planck Institute for Psychiatry, Martinsried, West Germany) presented his work on tissue-specific processing and modification of opioid peptide precursors. He reported that gene expression of the three opioid peptide systems (POMC, proenkaphalin A, and prodynorphin) varied between different tissues. Also, each of these opioid precursors can undergo tissue-specific post-translational processing, which enables the selective liberation of particular sets of biologically active opioid peptides from a precursor containing several opioid peptide sequences. This mechanism is exemplified by the processing of prodynorphin, where distinct opioid peptides with differential selectivities are derived from and within different neural and endocrine tissues. After enzymatic liberation from their respective precursors, neuropeptides can undergo post-translational modifications at the N- or C-termini. This may be of importance for the regulation of their biological activities. Seizinger presented preliminary information on the occurrence of two modifications, acetylation and amidation, which appear to be of physiological significance. He is now studying the roles of these modified neuropeptides in nervous system function.

R. Ivell and D. Richter (Institute for Physiological Chemistry, University of Hamburg, West Germany) reported on neuropeptide gene structure and expression in the mammalian hypothalamus and peripheral tissues. Neuropeptide hormones of the central nervous system are synthesized as parts of longer precursor polypeptides which are specifically cleaved and modified en route from the sites of synthesis in nerve cell bodies to sites of release in nerve terminals. These researchers, using molecular biology techniques, have identified the hypothalamic precursors to oxytocin, vasopressin, somatostatin, and luteinizing hormone-releasing hormone (LHRH) in rat and bovine tissue. Using the same approach, they have also identified the precursors to somatostatin (15,000 mol wt), vasopressin (19,000) and neuropeptide (12,000). Ivell and Richter have obtained as well mRNA and gene sequences for vasopressin and oxytocin precursors. In each gene the respective nonapeptide was found to occupy the first of three exons, the remaining exons encoding neurophysin moieties, and, in the case of the vasopressin precursor, a C-terminal glycopeptide of as yet unknown function. The oxytocin gene is also highly expressed in the corpus luteum of the midcycle bovine ovary. Although the transcribed sequence is identical to that in the hypothalamus, there is tissue-specific differential polyadenylation resulting in a shorter luteral message which did not, however, appear to influence protein product.

J.F. Rehfeld (Department of Clinical Chemistry, Rigsdospital, Copenhagen, Denmark) presented his work on the processing of preprocholecystokinin in the brain. Cholecystokinin (CCK) has a unique status among neuropeptides. It is the most abundant and widespread peptide system in the mammalian brain as well as being a highly potent neurotransmitter. Rehfeld found that, in accordance with the high tissue concentration in the cerebral cortex, there is a rapid and extensive translation and processing of the primary translation product (prepro CCK). The structure of prepro CCK in man, pig, and rat has been determined. In accordance with typical processing sites in the precursor, CCK was found to occur in multiple molecular forms in brain tissue. Characterization and quantitation of the different molecular forms indicated that the post-translational processing of prepro CCK follows at least three different pathways in the cerebral cortex. These

findings suggest that there are several types of CCK neurons in the brain with different transmitter products.

Specific Macromolecules in Cell-Cell Interactions in the Nervous System

This topic was covered in a symposium as well as oral communications and poster sessions at the ISN meeting, with emphasis on research dealing with N-CAM (neural adhesion molecule). N-CAM is a cell-surface glycoprotein that has been shown to mediate adhesion between cells that express it, including muscle and glial cells in addition to neurons. The binding involves homophilic interaction between N-CAMs on different cells and appears to be modulated by the sialic acid content of the molecule's carbohydrate moiety. Recent studies *in vitro* suggest that some of the key functions of N-CAM during development of the nervous system are associated with the establishment of normal pathways and projections.

U. Rutishuaser, J. Siler, and A.K. Hall (Case Western Reserve University, Cleveland, Ohio) in a collaborative study with F. Bonhoeffer (Max Planck Institute, Tübingen, West Germany) studied N-CAM in the visual system. They found that N-CAM-mediated binding appears to be required for neurite-neurite adhesions that maintain neighbor relationships among optic neurons. Furthermore, the selective expression of the molecule on the marginal endfeet of certain neuroepithelial cells suggests that this is an important factor in the guidance of retinal cell axons from the eye to the tectum. Studies by these researchers on the phylogeny of adhesion indicate that N-CAM function has been highly conserved among chordates and that N-CAM-like antigens also exist during certain stages of invertebrate development.

E. Bock (The Protein Laboratory, University of Copenhagen, Denmark) presented her studies on the biosynthesis of N-CAM. It was originally called D_2 when first discovered by Bock. She examined N-CAM in cultured fetal rat neuronal cells. Developmental changes in biosynthesis were studied in rat forebrain explant cultures. Bock found that two N-CAM polypeptides (A and B) were synthesized using radiolabeled precursors in cultured neurons. These polypeptides had different molecular weights, but both were found to contain only N-linked complex oligosaccharides and were polysialated. They were shown to be integral membrane proteins by radioiodination by photoactivation using hydrophobic labeling reagent. In rat forebrain explant cultures, N-CAM was synthesized as four polypeptides of varying molecular weights with similar patterns obtained on peptide mapping. Biosynthesis of two of these proteins increased with age relative to A and B. A and B were sulfated in embryonic brain, but sulfation was not detected at postnatal ages. Phosphorylation, on the other hand, of A and B was observed at all ages examined. Bock suggested that N-CAM function may be modified during development by changes in the relative synthesis of the different polypeptides as well as by changes in their glycosylation and sulfation.

C. Gorides, G. Gennarini, and M. Hirn (Center of Immunology, Institut National de la Santé et de la Recherche Medicale, CNRS, Marseille-Luming France) reported on the isolation and identification of a mouse brain surface antigen that appears to be identical with mouse N-CAM. It bears unusual 2-8 linked polysialic chains. During development, they found a pronounced decrease in sialic acid content, and they found that these changes could be correlated with changes in the adhesion properties of the molecules. The researchers have identified at least three distinct N-CAM, polypeptides in mouse brain and in mouse neural cell lines. Then polypeptides appear to be synthesized by three different mRNAs and differ mainly by the lengths of their cytoplasmic extensions. By screening an expression library with monoclonal antibodies they have cloned several N-CAM related cDNAs. These investigators are now involved in a precise study

of the biosynthetic and structural relationships between the various N-CAM polypeptides.

F.F. Rathjen (Institute for Developmental Biology, Tübingen, West Germany) described his studies on L1 antigen, which is involved in neural cell adhesion but is distinct from N-CAM. L1 and N-CAM were found to be distributed in different patterns in cerebellum from young postnatal mice. N-CAM was found to be present in all cell and neurite layers, whereas L1 was restricted to regions containing post-mitotic cells. These results indicate that two chemically and histochemically distinct cell surface polypeptides can contribute to the calcium-independent adhesiveness of neural cells.

Conclusion

The selection of research reports presented above shows that European scientists are using the latest techniques of immunology and genetic engineering to investigate nervous system function. These scientists are making important contributions to research in neurobiology with, in most laboratories, a smaller staff than is generally found in the US.

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